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The Controversy of Sunscreen Ingredients: Examining the Relationship Between Oxybenzone and Butylparaben on Stylophorum Pistillata

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Cover Page Footnote
We would like to acknowledge Professor Swaminathan for guiding us throughout the process of this research project. Your patience and constant reminder of our project timeline is one of the reasons why we are proud to present this research project. You affirmed confidence in us. Thank you for being with us every step of the way.

Introduction

Ultraviolet radiation has the capability to cause inflammation and different forms of skin cancer. To protect ourselves from the negative effects of the sun, we apply sunscreen. Sunscreen, however, can have harmful underlying consequences for organisms in the ocean, specifically coral. A select ingredient such as UV filters and preservatives in sunscreen have the capacity to cause unhealable coral bleaching in coral reefs around the world, threatening both animal and plant marine life. In our proposed research, we want to understand the relationship between select active ingredients of sunscreens and coral bleaching.

Background Information

Ultraviolet radiation (UV radiation) is a form of electromagnetic radiation that is emitted by the sun between the wavelengths of 100nm to 400nm. UV radiation can be classified into three types: UVA (320-400nm), UVB (290-320nm), and UVC (290-100nm) (Brunnings). These rays pose a problem to human health. UVC rays do not pose a significant threat because the rays are absorbed by ozone in the atmosphere. UVB and UVA rays, however, are responsible for creating DNA damage skin cancer, and inflammation of the epidermal and dermal layers. One of the major characteristics of UV rays on organisms is DNA damage. Figure one below depicts a representation of what occurs. When ultraviolet radiation is absorbed by DNA, the energy absorbed combines two nitrogenous bases (thymine and cytosine) into a dimer. A large collection of dimers in the DNA could result in irreparable damage within individual cells.

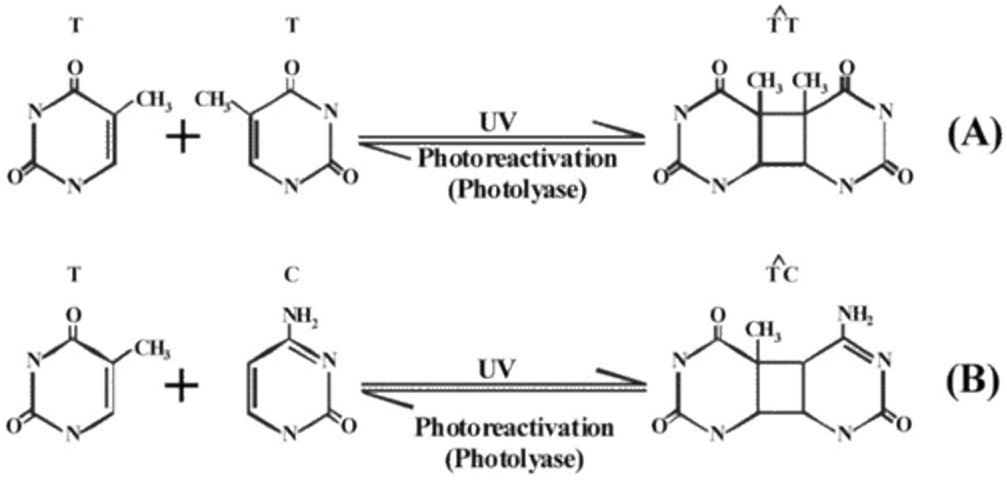


Figure 1. **UV radiation induces the creation of a Thymine dimer or a thymine cytosine dimer. Natural body mechanisms usually fix these dimers. If there is a long exposure to UV radiation, an accumulation of these dimers occurs, creating permeant DNA damage (Häder).**

To protect humans from UV rays, UV filters were created which absorb or scatter the rays innocuously. There are two different categories of UV filters- organic and inorganic. Organic UV filters are aromatic compounds with a carbonyl group and inorganic UV filters are titanium dioxide and zinc oxide. All UV filters aim to either reflect or absorb UV radiation into less harmful rays. Inorganic filters act as a physical barrier, reflecting and scattering incoming ultraviolet radiation. Organic filters, when confronted with photons, can respond in three ways: emit radiation at a higher wavelength, undergo conformational molecular changes, or release incident energy as heat (Lopes). This research will focus on the UV filter oxybenzone (BP-3) and the preservative butylparaben. Oxybenzone was chosen because it is one of the most common UV filters in sunscreens, lotions, and cosmetics (Downs). Butylparaben is a preservative that is also commonly found in personal care products, pharmaceuticals, and food products. There has been extensive research that showed that this preservative can cause estrogenic and endocrine disruptions on mammals but no research has been done to study the effect on coral (Danovaro).

Coral bleaching is caused by the loss and/or reduction of photosynthetic pigment concentrations of a symbiotic plant-like organism (algae) known as zooxanthellae. Zooxanthellae is responsible to provide corals with the essential nutrients through photosynthesis, and it also gives coral its vibrant color. However recent research has shown that chemicals in sunscreen such as parabens, cinnamates, and benzophenones play a fundamental role in harming coral, inducing stress-like conditions that ultimately force coral to expulse symbiotic zooxanthellae, facilitating coral bleaching (Rowe).

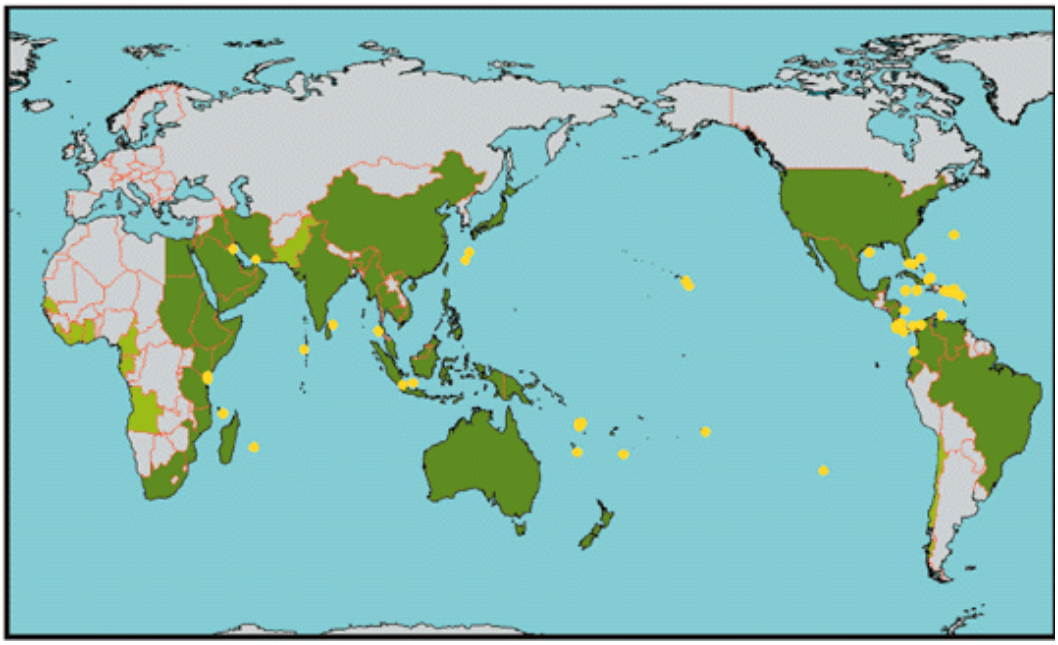


Figure 2. Yellow marks represent an increase in coral bleaching (Hinrichsen).

The frequency of bleaching disturbances has been increasing dramatically for the past four decades, and nearly all the major coral reefs (figure 2) have experienced some degree of coral bleaching (Buchheim). Coral Bleaching is expected to keep increasing since it is estimated that by the year 2025, 75% of the American population will be living in coastal areas (Hinrichsen).

Information about Oxybenzone

Oxybenzone is one of the major and commonly found UV filters in sunscreen that we will conduct research on. Oxybenzone, otherwise known as Benzophenone-3 (BP3) is an almost all-encompassing UV filter that is found in about 80% of all sunscreen and was detected by the Federal Centers for Disease Control and Prevention in 96% of the U.S population in the Summer months (Calaway).

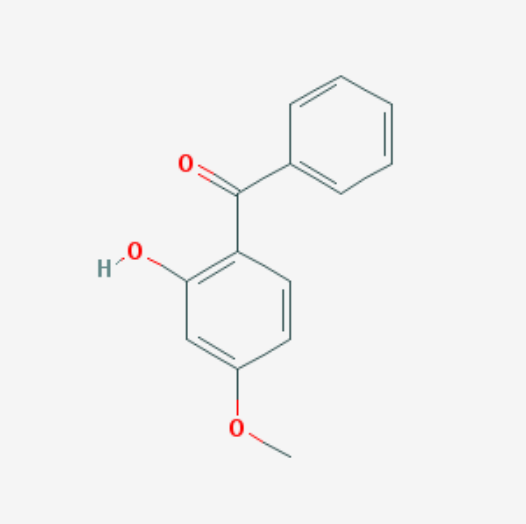


Figure 3. 2-D Structure of Oxybenzone (BP-3) (National).

As shown above in figure 3, oxybenzone belongs to a class of aromatic ketones and is used as a UV filter. Oxybenzone is highly valuable in sunscreen because it has the uncommon ability to protect the skin from both UVA and UVB light (Kruszelnicki). Although oxybenzone is an important UV filter to have, the chemical has vastly negative effects on organisms. Oxybenzone is a powerful hormone disrupter, increasing the amount of estrogen in both male and female organisms (Kruszelnicki). Oxybenzone also can cause DNA abrasions and facilitate vacuolation of cell bodies, nuclear membrane decay, and promote carcinogenic growth (Downs). In light, the effects of oxybenzone intensify

because it acts as a photo-toxicant: a chemical whose negative effects strengthen in light.

Information about Butylparaben

Butylparaben (figure 4), also known as Butyl 4-hydroxybenzoate, is part of the paraben family. Parabens are colorless, odorless, and tasteless crystals or white crystalline powders. Butylparaben is an organic compound used as a preservative in most foods and cosmetics (including sunscreens). This organic compound is soluble in alcohol, ether, glycerin, and propylene alcohol (International Journal of Toxicology). Research has linked butylparaben with estrogenic activity in males as well as female subjects. Its consumption causes irritation in skin, eyes, and in respiratory systems (National).

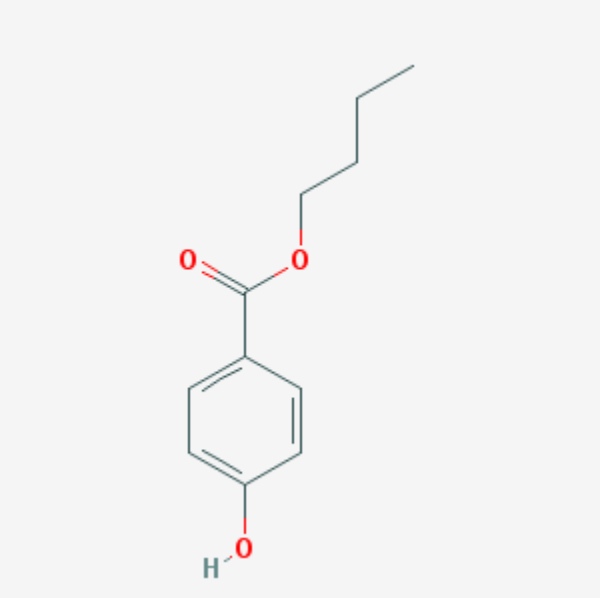


Figure 4. 2D Structure of Butylparaben (National).

Purpose of the Study

The purpose of this study will be divided into two separate components. The first component is to research an alternative (organic or inorganic) compound for oxybenzone. This compound must have similar UVA I, UVA II, and UVB absorption characteristics and have less lethal consequences towards coral. The second component is to determine the fatal extent of butylparaben towards coral. There have been studies on paraben and derivatives done in research laboratories such as Oxford, but its effect on coral is not sufficiently supported by an abundance of scientific journals and research (Boberg). A big part of this research is to conduct the first research on the relationship between butylparaben and coral bleaching, reaping invaluable data to the scientific community.

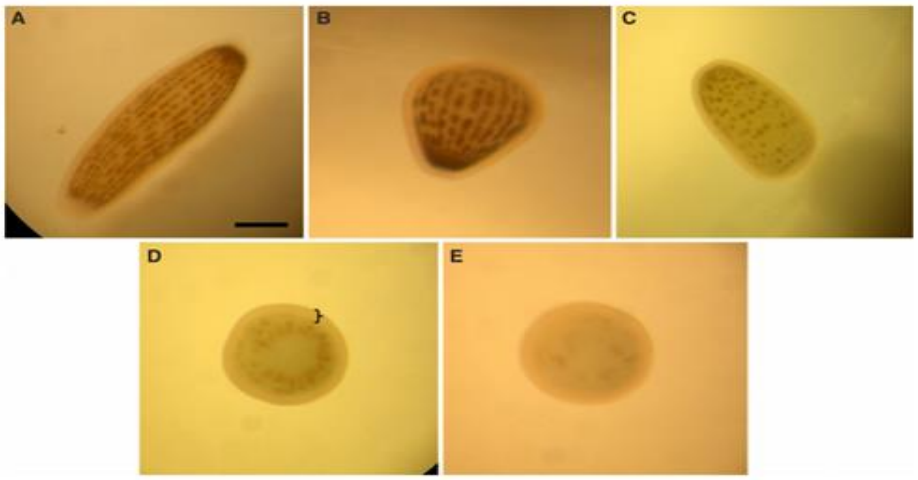
Review of the Literature

To develop this research, our team meticulously collected and studied similar research done by other scientists to understand their procedures and conclusions. Our team utilized the collected information about comprehensive information regarding oxybenzone and butylparaben to analyze the possible negative repercussions and create a research plan to collect data that could support or disapprove the previous results studied.

Oxybenzone

One study was done on the consequences of the UV filter Oxybenzone, known otherwise as Benzophenone-3 or BP-3, on coral planulae. The experiment was carried out a collection of scientists and researchers led by C.A. Downs. The UV filter Oxybenzone is a photo-toxicant which is a chemical whose negative effects worsen in light. The experiment this study conducted primarily focused on chlorophyll fluorescence, morphology, planula ciliary movement, and overall mortality. The coral species, Stylophora Pistillata was used as the subject of the experiment. Differing concentrations of BP-3 were tested, ranging from 228 parts per million to 2.28 parts per billion. Each concentration was conducted in both light and dark environments for a rotation of 8 hours and 24 hours.

Figure 5. **Letter A represents a healthy coral planula: an elongated figure and zooxanthellae**



(brown spots). As the concentration of BP-3 increased (from letters B-E) the morphology changed. The planulae became a dewdrop figure and became opaque (indicative of the expelling of zooxanthellae and bleaching). (Downs).

Figure 4 shows the change of morphology of the planulae as BP-3 concentrations increased. Figure A is the control. As the letters move sequentially, the oxybenzone concentration was increased. The organisms became sessile, as the overall shape of the planulae changed in a ball like structure. Bleaching was observed as the dark spots (collection of zooxanthellae) slowly disappeared as the planulae gradually became transparent

There were other relationships that were examined as the concentration of BP-3 increased. Chlorophyll fluorescence decreased, and cilia deformation and destruction became apparent on the epidermal layer of the cell. Tissue death was also common. There was also a positive relationship between "delamination of the nuclear membrane and vacuolization of the inner nuclear membrane" (Downs). In the zooxanthellae chloroplast degradation and thylakoid membrane degradation were apparent. DNA AP lesions were also exposed in light as well as DNA degradation (thymine dimers were found). Mortality also increased as concentration increased. In conclusion, this research stated studied the relationship between BP-3 and the lethal effects on the planulae (Down).

In another study done by the Department of Marine Sciences and Department of Chemical Sciences and Technologies at the Polytechnic University of the Marche in Italy, sunscreens containing the ingredients benzophenone-3 were tested on coral to examine the consequences on species of corals. The experiment was done with four different concentrations of sunscreen (10 μL,33 μL,50 μL,100 μL per L of artificial sea water) on two different species of coral, Stylophora pistillata and Millepora complanata. The results showed that coral bleaching was the prominent result in all four of the concentration, even observed in the lowest concentration. Large amounts of coral mucus (zooxanthellae and coral tissues) were released. The rates of bleaching were faster in large quantities of BP-3.

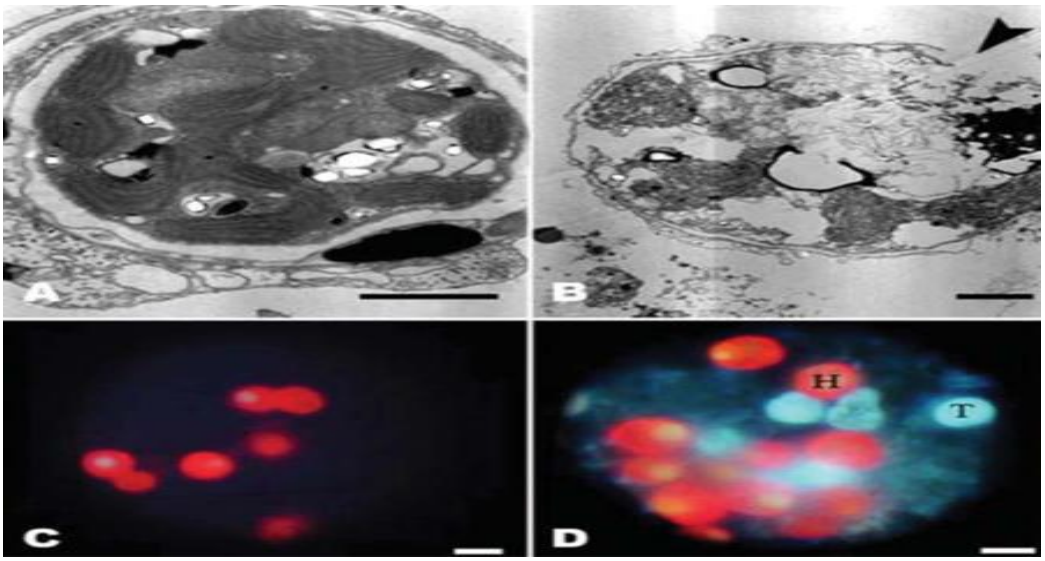


Figure 6. Letter A shows a healthy planula where there are an abundance of dark spots (zooxanthellae). In B, BP-3 concentrations were introduced. Large white spots were present, indicative of bleaching. Letters C and D represents a before concentration planulae and after concentration, as an autofluorescence image. The red spots are healthy zooxanthellae while the blue are the damaged or partially damaged organisms. (Danovaro).

Figure 6 shows planulae before and after being exposed to BP-3. Letters A and B were taken with an electron microscope and C and D were taken as an auto fluorescence image. The transition from A to B and C to D clearly shows a decreased level in the number of zooxanthellae (Danovaro).

Butylparaben

Several studies have been done to understand the effects of butylparaben in humans as well as animals. One of the case studies was done using human skin In Vitro (Table 1), and it demonstrated that at least a portion of this chemical is absorbed and retained in body tissues before the chemical undergoes hydrolysis. The experiment also correlated that an increase in temperature enhanced transdermal penetration, and it showed intensified dermatitis in many of the membrane models.

Model Membrane	Comments	Reference
Human epidermis in Franz cell diffusion expts. with receptor fluid at 23-45 °C	Transdermal flux from a saturated aqueous solution and epidermal retention increased with increasing temperature. More butylparaben than methylparaben was retained in the epidermis. Amounts increased with higher temperature while the transdermal flux of methylparaben was greater than that of butylparaben. At 37 °C, estimated epidermal diffusivity for methylparaben was $26.54 \pm 4.17 \times 10^{-4}$ cm/s compared to $1.52 \pm 0.22 \times 10^{-4}$ cm/s for butylparaben.	Akomeah et al. (2003 poster, 2004)
Human skin	Fluxes from cosmetic emulsions decreased with increasing chain length.	Dal Pozzo and Pastori (1996)
Human callus	The higher the octanol-water partition coefficient, Kow, the greater the binding to keratinized structures of the stratum corneum. Skin penetration was greater when the compound was highly soluble in the vehicle.	Hagedorn-Leweke and Lippold (1998)
Human erythrocyte ghost membranes	Absorption increased with higher lipophilicity.	Lee and Kim (1994)
Different human skin layers	Four carboxylesterases from skin, subcutaneous fat, and blood had different hydrolyzing capabilities for the parabens at pH 8.0. No hydrolysis at pH 5.0.	Lobemeier et al. (1996)

Table 1. In Vitro skin penetration studies examples (Hinrichsen).

In another In Vitro study, researchers used guinea pigs' skin to understand the percutaneous absorption of butylparaben (Table 1). The amount of compound that was absorbed by the skin depended on the partition coefficient of the system. The experiment concluded that solubilizes like propylene glycol and PEG 400 were found to increase antimicrobial activity and decrease absorption. L-menthol component of ethanol, as well as d-limonene decreased the absorption of butylparaben significantly.

Model Membrane	Comments	Reference
Rat skin	Ninety-six percent of the penetrated amount of butylparaben was metabolized in the skin to 4-HBA.	Bando et al. (1997)
Rat skin	The relationships among lipophilicity, metabolic rate, and skin permeation were studied. Cutaneous metabolism in the viable layer is important for butylparaben.	Seko et al. (1999)
Guinea pig dorsal skin	Certain agents and vehicles altered permeability.	Kitagawa et al. (1997)
Guinea pig dorsal skin	Addition of Polysorbate 80 or polyethylene glycol 410 reduced penetration of butylparaben in water.	Komatsu and Suzuki (1979; cited by CIR, 1984)
Guinea pig skin	Penetration of butylparaben from liposomes was studied.	Komatsu et al. (1986)
Guinea pig skin	*d*-Limonene and oleic acid enhanced penetration.	Koyama et al. (1994)
Guinea pig skin	Cyclodextrin complexation decreased percutaneous absorption.	Okamoto et al. (1986)
Guinea pig skin	Skin penetration enhancers increased accumulation in skin.	Okamoto et al. (1991)
Guinea pig skin, two-layer	1-Geranylazacycloheptan-2-one increased skin penetration from an aqueous vehicle.	Yamashita et al. (1993)
Rabbit corneas	The relationship between corneal permeability and lipophilicity was studied.	Lee et al. (1991)
Snake skin	Smaller lipophilic compounds penetrated the skin more readily.	Itoh et al. (1990)
"Idealized skin model membranes" [silastic and demeticone were indexed]	Effects of alcoholic solvents on skin permeation kinetics were studied.	Twist and Zatz (1986)

Table 2. Reporting the permeability of butylparaben through guinea pig, rat, rabbit, and snake skin (Hinrichsen).

Purpose Based Literature

To formulate what each component of the research should encompass, we researched and studied previously done research that could help us create our experiment.

The first component of the experiment is to test which derivative of oxybenzone has the least negative effect on coral. We based this component off a study done by the University of Sao Paulo. The university conducted research on four different derivatives of oxybenzone: BP-3 phenylamine, BP-3 methoxy-phenylamine, BP-3 carbazole, and BP-3 carbonate to see which derivative had the same UV absorbing strength as BP-3 but was less toxic. The purpose of this study was to find an alternative to BP-3 used in cosmetics and sunscreen. The research concluded that BP-3 phenylamine and BP-3 methoxy-phenylamine were the best considered to be alternatives because both achieved similar or higher absorption of both UVA I/UVA II and UVB (Maria). Both derivatives were not considered phototoxic as well. Based on this research, the first component of the experiment would be to comparing BP-3, BP-3 phenylamine and BP-3 methoxy-phenylamine on coral.

The second component of the experiment is to research the effects of butylparaben in coral species. Butylparaben is one of the various components of sunscreen, and by being in contact with skin the spreading of this chemical to coral system is eminent. We based the second component of our research off a research done by a collaboration of the Research Triangle Park and the Integrated Laboratory System. The Review of Toxicological Literature of butylparaben, prepared by the Research Triangle Park, and the Integrated Laboratory System reported numerous research done, in humans and in animals (mammals specifically), that have proven different degrees of hormonal activity from butylparaben exposure. Most of the results concluded that constant exposure of

butylparaben cause negative effects in reproductive systems of mammals, but there is lack of research that involves aquatic systems such as coral. The purpose for this study is to identify the effects that butylparaben have specifically in coral systems, and if it is a harmful chemical that species (Masten).

Materials and Procedures for Experimentation Component 1

- Stylophora pistillata Planulae (Count 100)
- Planula Traps
- National Park Authority Permits
- Fisher Scientific Environmental-Grade Water
- Sigma -Aldrich Sea Salts
- Oxybenzone (solid soluble)
- Molecular Dynamics Microplate Fluorimeter
- Dojindo DNA damage Quantification KIT-AP Site Counting
- Transmission Electron Microscope
- Culture Plates/Dishes
- Probing Instruments (Glass Rods)
- Transparent/Opaque Bins
- Transparent/Opaque Covering

****To emulate the experiment done by C.A. Downs (Page 7). This experiment will test the effects of derivatives of oxybenzone, the procedures will be relatively similar.**

Procedures:

1) Obtain Stylophora pistillata from a colony.

2) Use buoyant traps that are 25cm in diameter to collect planula. Inspect planulae for any abrasions or physical infections/disorders. Collect around 100 planulae over a course of 2-3 days.

3) Create all seawater (ASW) artificially using fisher scientific environmental-grade water and sigma-aldrich sea salts. Use the sea salts to carefully measure and optimize the sea water to a salinity of 38ppt in 22 degree celsius temperature.

4) Obtain BP-3 and collect stock and exposure by making BP-3 soluble in dimethyl sulfoxide and diluted ASW.

5) Create concentrations of BP-3 of: nine solutions each.

- 1mM BP-3 (228 parts per million) (9 Count)
- 0.1mM BP-3 (22.8 parts per million (9 Count)
- 0.01 mM BP-3 (2.28 parts per million) (9 Count)
- 0.001 mM BP-3 (228 parts per billion) (9 Count)
- 0.0001 mM BP-3 (22.8 parts per billion) (9 Count)
- 0.00001 mM (2.28 parts per billion) (9 Count)

6) Place one planulae that was gathered in steps 1-2 in each of the solutions of each concentration.

7) Place 3 solutions of each concentration in each of the three bins.

8) Label one bin “24h”, one bin “8h in dark”, and one bin “8h in light”

9) Place the transparent/opaque covering over the “8h in dark” bin so no light seeps through.

10) Place the “8h in light” bin next to the “8h in dark” bin but exposed to a light source.

11) After 8 hours, carefully extract each planulae of the light bin and into a culture plate. Repeat with the dark bin with different culture plates. Keep the planulae in the light and dark separated.

Move on to data analysis

12) Put the remaining planulae in the 24h bin and place the bin exposed to a light source. Place the bin in a light source for 12 hours. After 12 hours, place the opaque/transparent covering over the bin to make sure no light enters the bin. After the remaining 12 hours carefully extract each planulae into its own individual culture plate.

Move on the data analysis

Data Analysis

*Chlorophyll Fluorescence = estimate of bleaching

Use molecular dynamics microplate fluorometer (with excitation wavelength of 445 nm and emission wavelength of 685) to collect data for each planulae culture plate. Record data.

*DNA Abasic Lesions

With the Dojindo DNA Damage Quantification Kit-AP, collect information for each planulae and record the data. Make notes and observations for the size and intensity of DNA abrasions.

*Transmission Electron Microscopy

Use the transmission electron microscope to evaluate and record each image of each planulae. Make notes and observations of the size and disfiguration of planulae.

Materials and Procedures for Experimentation Component 2

- Water
- Butylparaben
- Experimental Tanks: range from 10 gallons down to 0.5 gallons
- Lightening: 54 W T5 fluorescent, 20W T12 fluorescent, 24 W T5 fluorescent, 55W power compact (PC) fluorescent, 26 W PC fluorescent, 1000 W metal halide, 400 W metal halide, 250W metal halide, 9 W PC fluorescent, 150W metal halide, 12 W LED, 5 W compact fluorescent (CFL)
- Timers: can be used to control light duration
- Power Heads: to keep the water flowing
- Filtration
- Aquarium heaters
- Thermometers
- Stylophora pistillata or Favia favus
- Coral Food

Procedure:

Since this is the first experiment that focuses on the effects of butylparaben in coral systems, there will be only one variable. The concentrations of butylparaben in a constant system. The development, and corrections of the procedures, will be specified as continued

Water testing. Needed to avoid cross contamination, do it twice a week. Performing full water exchanges one to three times a week. APPENDIX G for guidelines.

Calibration of Salinity: Deionized water should be slowly added directly to the tank in an area of high water flow to adjust salinity.

Measure the pH of the water every week.

Calcium: Calcium adjustments can be made with known concentrations of either anhydrous calcium chloride or calcium chloride dihydrate solutions (~60 g/L of either)

Alkalinity: Alkalinity levels can be adjusted using known concentrations of a sodium bicarbonate (i.e., baking soda) solution (60 g/L)

Magnesium concentration control. No need to repeat this procedure.

Ammonia, Nitrite, Nitrate, and Phosphate: Reduce this chemical since it's harmful for coral. Increased frequency or volume of water changes. Test it weekly.

Feeding: powdered food is preferable. Feeding is only applied for experiments longer than 2 weeks. It needs to be rinsed with Deionized water to avoid cross contamination. Feeding once per week.

Acclimation of coral: slowly dripping system water (water from the holding or quarantine tank in which the new coral will be placed) into a separate container, usually a 5-10-gallon tank, containing the coral and enough shipment water to cover them. Appendix H for set up.

Butylparaben: Starting off by low dosage of butylparaben, increase it every 3 weeks.

Future Research

Further research can be implemented after this study. If both component 1 and component 2 of the experiment are successful (oxybenzone derivatives are as efficient as BP-3 and less lethal and butylparaben are found to be not as harmful) then a new research idea could be to combine pieces of both component 1 and 2. The next step is to test the effects of a combination of the successful oxybenzone derivative and differing concentrations of butylparaben on coral life and bleaching. This research would show the relationship between butylparaben and oxybenzone and have the capability to provide relatively innocuous alternative ingredients to the active ingredients used in pharmaceutical sunscreen.

Significance

The significance of this experiment is to discover a new product that could both protect humans from dangerous UV radiation and have little to no negative effect on coral. Coral Reefs contribute to a large component of many coastal economies in the form of tourism while providing sustainable habitats for an abundance of marine life. Discovering a mixture or compound that could shield UV rays and is sustainable will aid in the recovery in the coral reefs and benefit both humans and marine ecosystems.

Budget

Given that previous research conducted by Department of Marine Sciences and Department of Chemical Sciences and Technologies at the Polytechnic University of the Marche in Italy, was held in Red Sea, Caribbean Sea, the Indian Ocean (off of Thailand), and the Pacific Ocean near Indonesia, the research will focus on Australia's Great Barrier Reef, Key West, Florida and Los Angeles, California. Australia's Great Barrier Reef is 60% bleached, and 5% damaged as of 2002 (gbrmpa.gov.), which gives this reef a significance in our research. The research will also be conducted in Key West, Florida and Los Angeles, California,

which are two of the nation's most populated and tourist magnet destinations. Due to high volumes of tourists, surely there will be appropriate data from the research which will support how oxybenzone and butylparaben cause coral bleaching.

In order to carry out our procedure, our team needs the appropriate scientific instruments and aid to produce credible results. First and foremost, this experiment requires a lab, which will be used for a duration of six months in each location. Renting a lab will cost about $40,000 per month for six months given that there will be six people working together. (Appendix A) The research lab will allow for our team to further study the levels of damage each concentration of oxybenzone and butylparaben cause. Furthermore, a research boat will also be needed to maneuver out in the ocean and collect the organisms. Given that our team will consists of six people, a boat that will be able to fit us will be around $3,000 per week, just for rental. During the duration of six months the four people who will work alongside us will be a marine biologist, University Biology Student, scuba diver, and a coral expert. The salary for each aid will be around $20,000 per month, and $60,000 for each researcher. To acquire the materials listed for each research (Appendix B), the cost will be around $350,000. Some instruments such as the Transmission Electron Microscope can be rented, but it is preferred to have them purchased brand new, around $100,000, just so we will be able to use it in the other research locations. Other equipment need to be scientific graded for accuracy and precision. However, there should also be room for error in this budget, and considering possible damages and alterations in the experiment.

Appendix

Appendix A

Class 1, ~228 square feet (typically 2 to 4 people), $18,240 for four months

Class 2, ~314 square feet (typically 3 to 6 people), $25,120 for four months

Class 3, ~251 square feet (typically 2 to 5 people), $20,080 for four months

Class 4, ~291 square feet (typically 3 to 5 people), $23,280 for four months

Appendix B

- Stylophora pistillata Planulae (Count 100)
- Planula Traps
- National Park Authority Permits
- Fisher Scientific Environmental-Grade Water
- Sigma -Aldrich Sea Salts
- 200g of BP-3 (solid soluble)
- Molecular Dynamics Microplate Fluorimeter
- Dojindo DNA damage Quantification KIT-AP Site Counting
- Transmission Electron Microscope
- Culture Plates/Dishes
- Probing Instruments (Glass Rods)
- Transparent/Opaque Bins
- Transparent/Opaque Covering
- Water
- Butylparaben
- Experimental Tanks: range from 10 gallons down to 0.5 gallons
- Lightening: 54 W T5 fluorescent, 20W T12 fluorescent, 24 W T5 fluorescent, 55W power compact (PC) fluorescent, 26 W PC fluorescent, 1000 W metal halide, 400 W metal halide, 250W metal halide, 9 W PC

fluorescent, 150W metal halide, 12 W LED, 5 W compact fluorescent (CFL)

- Timers: can be used to control light duration
- Power Heads: to keep the water flowing
- Filtration
- Aquarium heaters
- Thermometers
- Stylophora pistillata or Favia favus
- Coral Food

Appendix C

Lab (Class 1, ~228 square feet (typically 2 to 4 people): $18,240 for four months
http://www.mbl.edu/research/whitman-center/lab-fees/

Oxybenzone (200 g): $ +16.40
http://www.makingcosmetics.com/Oxybenzone_p_262.html

Butylparaben (125 g): $ 89.45
https://www.grainger.com/search?searchQuery=Butylparaben&adgrpID=19675741274&kwdID=%2Bbutylparaben&cm_mmc=PPC:+Google+PPC&s_kwcid=AL!2966!3!92187136274!b!!g!!%2Bbutylparaben&ef_id=V7tevgAABec4WS2e:20170324000020:s

Marine research boat rental (V-39, Elis Olsson Monarch-29'): $ 75.00/hr ($75.00/hour (Large Vessel Reservation; minimum crew of two at $55.00/hour each.)

-Gasoline: market price

-trailer rental: $20/day
http://www.vims.edu/about/leadership/sponsored_programs/apply/rates/vessels.php

Transportation (Pick-up): $1383.50/month (x4)

https://www.enterprise.com/en/reserve.html#cars

Lodging (Miami Marriott Dadeland): Price for 3 adults for 30 nights: $10,140 (x4)

http://www.booking.com/searchresults.html?aid=336408&label=miami-yOw_DkZ7r0sNwBR9DOhDVAS154563832741%3Apl%3Ata%3Ap1300%3Ap2%3Aac%3Aap1t1%3Aneg%3Afi%3Atikwd-35025360%3Alp9008185%3Ali%3Adec%3Adm&sid=35f2cdbe2f45a14194a93cd748606fb4&sb=1&src=city&src_elem=sb&error_url=http%3A%2F%2Fwww.booking.com%2Fcity%2Fus%2Fmiami.html%3Faid%3D336408%3Blabel%3Dmiami-yOw_DkZ7r0sNwBR9DOhDVAS154563832741%253Apl%253Ata%253Ap1300%253Ap2%253Aac%253Aap1t1%253Aneg%253Afi%253Atikwd-35025360%253Alp9008185%253Ali%253Adec%253Adm%3Bsid%3D35f2cdbe2f45a14194a93cd748606fb4%3Binac%3D0%26%3B&ssne=Miami&ssne_untouched=Miami&city=20023181&checkin_month=6&checkin_monthday=21&checkin_year=2017&checkout_month=7&checkout_monthday=21&checkout_year=2017&sb_travel_purpose=business&room1=A%2CA%2CA&no_rooms=2&group_adults=3&group_children=0

National park fees: $400/day

Fisher scientific environmental-grade water: $100.96-235.22

https://www.fishersci.com/shop/products/water-environmental-grade-fisher-chemical-2/p-216445

Sigma -Aldrich sea salts (1kg): $82.70

http://www.sigmaaldrich.com/catalog/product/sigma/s9883?lang=en&region=US

Dojindo DNA damage Quantification KIT-AP Site Counting: $2,695

Fluorometer: $ 2,550

https://www.thermofisher.com/us/en/home/industrial/spectroscopy-elemental-isotope-analysis/molecular-spectroscopy/fluorometers/qubit.html?gclid=CNiV84u17tICFQaBswodjyYBZw&

s_kwcid=AL!3652!3!109132858031!b!!g!!%2Bdna%20%2Bquantification&ef_id=V7tevgAABec4WS2e:20170324053224:s

Transmission Electron Microscope(rent): $45.75/hr

https://www.scienceexchange.com/browse?category=electron-microscopy&utm_source=google&utm_medium=cpc&utm_campaign=Electron_Microscopy&utm_content=transmission_electron_microscopy&utm_term=%2Btransmission%20%2Belectron%20%2Bmicroscopy&creative=49467539870&keyword=%2Btransmission%20%2Belectron%20%2Bmicroscopy&matchtype=b&network=g&device=c&gclid=CMynl7q27tICFZKFswod-SoPjw

Culture plates/dishes (10 pack): $19.75

http://www.carolina.com/catalog/detail.jsp?prodId=821862&s_cid=ppc_products&utm_source=google&utm_medium=cpc&s_cid=ppc_gl_products&scid=scplp821862&sc_intid=821862&gclid=CNbpw4e37tICFdiKswodp0cLQQ

Probing instrument: $468.00

https://www.coleparmer.com/i/ysi-pro-10-pro-10-ph-orp-temperature-portable-meter/5935216?PubID=UX&persist=true&ip=no&gclid=CLP50bu37tICFUSBswodWrwA6Q

Storage bins: $9.97 each

http://www.homedepot.com/p/HDX-27-Gal-Storage-Tote-in-Black-HDX27GONLINE-5/205978361

Experimental Tanks: range from 10 gallons down to 0.5 gallons: $300-400

https://www.walmart.com/ip/Aquaculture-10-Empty-Aquarium/144433503?wmlspartner=wlpa&selectedSellerId=0&adid=22222222227039953670&wl0=&wl1=g&wl2=c&wl3=90994642832&wl4=pla-184879976672&wl5=9008185&wl6=&wl7=&wl8=&wl9=pla&wl10=8175035&wl11=online&wl12=144433503&wl13=&veh=sem

Lightening: 54 W T5 fluorescent, 20W T12 fluorescent, 24 W T5 fluorescent, 55W power compact (PC) fluorescent, 26 W PC fluorescent, 1000 W metal halide, 400

W metal halide, 250W metal halide, 9 W PC fluorescent, 150W metal halide, 12 W LED, 5 W compact fluorescent (CFL): $6.27, $2.69, $20.45, $26.99, $12.91, $15.67, $10.24, $9.18, $12.99, $11.32, $17.13, $2.32

Timers:

can be used to control light duration: $8.79

Power Heads: to keep the water flowing: $285

Filtration: $200.00

Aquarium heaters: $24.00

Thermometers: $34.00

Stylophora pistillata or Favia favus

Coral Food: $15

Food: $4,000

Emergency: $5,000

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